Experiment AM-7: Crayfish Heart

Equipment Required

PC or Mac Computer

IXTA, USB cable, IXTA power supply

FT-302 Force transducer

Thread

Insect pin hook

Ring stand and clamp

Dissection dish deep enough to submerge a crayfish

Dissecting tools and #2 insect pins

Crayfish Ringer's Solution (See appendix)

10^{-3}M Serotonin in Ringer's (See appendix)

10^{-3}M GABA in Ringer's (See appendix)

FT-302 Force Transducer Setup

1. Locate the FT-302 force transducer and plug it into Channel A5.

Figure AM-7-S1: The FT-302 force transducer connected to the IXTA.

Calibration of the FT-302 Force Transducer

1. Type **No Weight** in the Mark box. Click Record, and click the Mark button. Record for ten seconds with no weight hanging from the arm or hook of the transducer.
2. Type **5 grams** in the Mark box. Hang a 5 gram weight on the arm or hook of the transducer. Click the Mark button. Record for ten more seconds.
3. Click Stop to halt the recording.
4. Select Save As in the File menu, and name the file. Choose a destination on the computer in which to save the file. Click on the Save button to save the data file.

Unit Conversion

1. Scroll to the beginning of data when no weight was attached to the force transducer.
2. Use the Display Time icons on the LabScribe toolbar to adjust the display time of the Main window to show the complete calibration data on the Main window.
3. Click the Double Cursor icon so that two vertical cursors appear on the Main window. Place one cursor on the flat section of data collected when no weight was attached to the force transducer, and the second cursor on the flat section of data collected when the 5 gram weight was attached to the transducer.
4. To convert the voltages at the positions of the cursors to correct values, use the Simple Units Conversion dialogue window. Click V2-V1 on the force channel, then select Units, and select Simple.

    - Put a check mark in the box next to Apply units to all blocks. Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - Enter “Zero” in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter “5” in the box to the right of the corresponding voltage recorded when the 5 gram weight was hung on the hook of the transducer.
    - Enter the name of the units, grams, in the box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

In the 10 gram range, the FT-302 will deliver approximately 75 mV/gram at x1 gain and approximately one tenth of that in the 100 gram range. The FT-302 is now ready for use.

Experiment AM-7: Crayfish Heart

The Dissection

1. Cover a crayfish with ice for 5 minutes.
2. With scissors, remove the claws, walking legs, and abdomen ("tail") from the crayfish.
3. The crayfish heart is located dorsally, at the posterior end of the thorax. Use the scissors to carefully remove a small section of carapace over the heart. The hypodermis, the red membrane directly beneath the carapace, should also be removed.
4. The beating yellow-white ventricle of the heart should be clearly visible.
5. Use four #2 insect pins to pin the crayfish securely into a wax or Sylgard dish.
6. Cover the crayfish with 100 ml of room temperature Ringer's. If 100 ml of saline doesn't cover the crayfish, use more. It is important to keep track of exactly how much saline is in the dish.

The Preparation

1. Place the dissection dish so that the crayfish ventricle is directly below the end of the transducer. The force transducer should be about 15 cm above the heart, with the blade of the transducer being horizontal.

Figure AM-7-L1: The crayfish heart preparation

2. Bend a metal pin to form a hook. Tie a 20 cm length of thread behind the head of the hook.
3. Push the hook through the ventricle wall until the bend of the hook is inside the heart.
4. Tie the loose end of the thread to the hole in the blade or on the hook of the transducer. Loosen the clamp holding the transducer and gently raise it on the ring stand. Put enough tension on the thread to raise the ventricle very slightly.

Figure AM-7-L2: Force transducer hook placed through the ventricle of the heart.

Warning: The heart preparation used in this experiment is functional for a limited period of time. Keep the heart muscle covered in saline. To conserve time, complete all the exercises in the experiment before analyzing the data.

Exercise 1: The Heart Rate

Aim: To record the mechanical trace produced by the contraction of a resting heart, and to determine the resting heart rate.

Approximate Time: 30 minutes (including dissection)

Procedure

1. Type **Resting** in the Mark box.
2. Click the Record button and then the Mark button to attach the comment to the record. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the heart contractions for thirty seconds. A sample recording is shown below.
4. Click Stop to halt the recording.

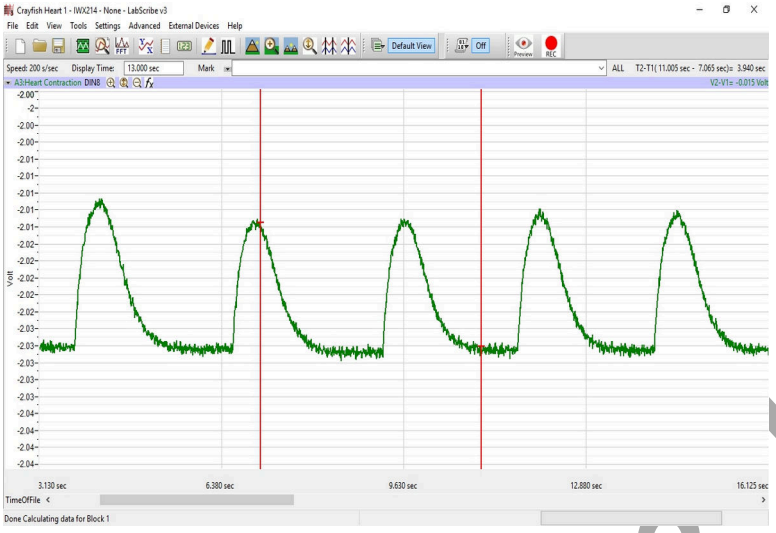

Figure AM-7-S3: Recording of the contractions of the crayfish heart.

5. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file, like your lab group folder.

Exercise 2: Effects of Cold Temperature

Aim: To record changes in heart rate after the heart is bathed in cold Ringer's solution.

Approximate Time: 15 minutes

Procedure

1. Type **Room Temp Ringer's** in the Mark box.
2. Click the Record button and the Mark button to attach the comment to the recording. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the heart contractions for thirty seconds.
4. Click Stop to halt the recording.
5. Draw off the room temperature Ringer's with a syringe, and add chilled saline, taking care to replace the saline with the same volume as was drawn off.

Warning: It is important to keep the saline at the same depth throughout the experiment, as the depth of the saline will affect the amplitude of the recorded beats. The dissecting dish should also be firmly affixed to the table with clay so that it won't be accidentally bumped, changing the tension on the ventricle.

6. Type **Cold Ringer's** in the Mark box.
7. Click the Record button and then the Mark button to attach the comment to the recording.
8. Record until the heart has recovered from the effects of cold Ringer's solution.

Note: *Recovery is when the amplitude and rate of the heart contraction have returned to the resting values.*

9. Click Stop to halt the recording.
10. Select Save in the File menu.

Exercise 3: Effects of Drugs

Aim: To monitor the effects of Serotonin and GABA on the amplitude and rate of heart contraction.

Approximate Time: 45 minutes

Procedure-Serotonin

1. Type **Pre-Serotonin** control in the Mark box.
2. Click the Record button and then the Mark button to mark the recording. Click AutoScale to increase the size of the deflection on the Main window.
3. Record the heart contractions for thirty seconds.
4. Type **Serotonin 10^{-6}M** in the Mark box.
5. Add 100 microliters of the 10^{-3}M stock Serotonin solution to the saline in the dish. Gently stir the saline to disperse the Serotonin.

Note: *If you used a volume other than 100 ml, adjust the amounts of the stock solution accordingly. For example, if you covered the crayfish with 150 ml, add 150 microliters of the stock Serotonin solution to create a 10^{-6}M Serotonin solution.*

6. Click Record to start the recording, and click the Enter key on the keyboard.
7. Record the effects of 10^{-6}M Serotonin for two minutes.
8. Click Stop to halt the recording.
9. Add an additional 900 microliters of the 10^{-3}M Serotonin solution to the saline in the dish to create a 10^{-5}M solution. Gently stir the saline to disperse the Serotonin throughout the saline.
10. Type **Serotonin 10^{-5}M** in the Mark box.
11. Click Record to start recording and click the Mark button.
12. Record for two minutes.
13. Click Stop to halt the recording.
14. Add 10 ml of the 10^{-3}M Serotonin stock solution to the saline in the dish to create a 10^{-4}M Serotonin solution.

15. Type **Serotonin 10^{-4}M** in the Mark box.
16. Click Record and then the Mark button.
17. Record for two minutes.
18. Click Stop to halt the recording.
19. Replace the saline with 100 ml of fresh saline, and allow the heart to recover.

Note: *It is possible that the heart will not return to pre-treatment conditions. In this case, wait until the heart has come to a new steady-state, and record the heartbeat at that time. This should occur within ten minutes after the saline change.*

20. Type **Post-Serotonin** recovery in the Mark box.
21. Once the heart has recovered, click Record and then the Mark button.
22. Record for thirty seconds.
23. Click Stop to halt the recording.
24. Select Save in the File menu.

Procedure-GABA

1. Type **Pre-GABA** control in the Mark box.
2. Click Record and then the Mark button
3. Record for thirty seconds.
4. Click Stop to halt the recording.
5. Add 100 microliters of the 10^{-3}M stock GABA solution to the saline in the dish. Gently stir the saline to disperse the GABA throughout the saline.
6. Type **GABA 10^{-6}M** in the Mark box.
7. Click Record and then the Mark button.
8. Record the heart contractions for two minutes.
9. Click Stop to halt the recording.
10. Add an additional 900 microliters of the 10^{-3}M GABA solution to the saline in the dish to create a 10^{-5}M solution. Gently stir the saline to disperse the GABA throughout the saline.
11. Type **GABA 10^{-5}M** in the Mark box.
12. Click Record and then the Mark button.
13. Record for two minutes.
14. Click Stop to halt the recording.

15. Add 10 ml of the 10^{-3}M GABA stock solution to the saline in the dish to create a 10^{-4}M GABA solution. Gently stir the saline to disperse the GABA.
16. Type **GABA 10^{-4}M** in the Mark box.
17. Click Record and then the Mark button.
18. Record for two minutes.
19. Click Stop to halt the recording.
20. Replace the saline with 100 ml of fresh saline, and allow the heart to recover.
21. Type **Post-GABA** recovery in the Mark box.
22. Once the heart has recovered, click Record and then Mark.
23. Record for thirty seconds.
24. Click Stop to halt the recording.
25. Select Save in the File menu.

Data Analysis

Exercise 2: Temperature Effects

1. Scroll to the data recorded from the heart fifteen seconds before cold Ringer's solution was added to the heart. Click the AutoScale button to maximize the size of the heart contractions on the window.
2. Use the Display Time icons to adjust the Display Time of the Main window to show five contractions on the Main window. The contractions can be selected by:
    - Placing a cursor before the first contraction, and a cursor after the fifth contraction; and
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the five selected contractions to the width of the Main window.
3. Data can be collected from the Main window or the Analysis window. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.
4. The mathematical functions and values, V2-V1 and T2-T1 are shown for each channel of data
5. Maximize the height of the trace on the Heart Contraction Channel by clicking the AutoScale All button on the toolbar.
6. Once the cursors are placed in the correct positions for determining the amplitude and period of each heart contraction, the values of the parameters in the Function Table can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal, or on a separate data table.

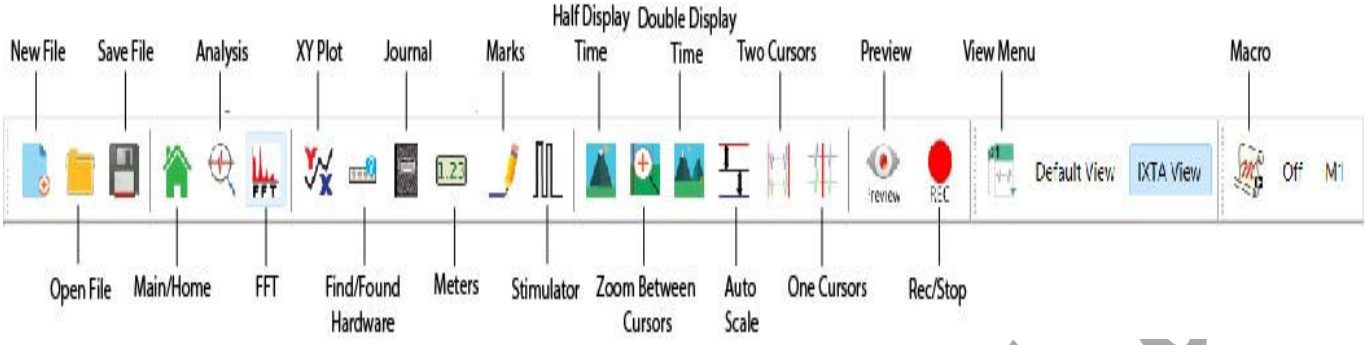

Figure AM-7-L4: LabScribe Toolbar

7. The functions in the channel pull-down menu of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:
    - Place the cursors at the locations used to measure the amplitude and period of each heart contraction.
    - Transfer the names of the mathematical functions used to determine the amplitude and times to the Journal using the Add Title to Journal function in the Heart Contraction Channel pull-down menu.
    - Transfer the values for the amplitude and period to the Journal using the Add Ch. Data to Journal function in the Heart Contraction Channel pull-down menu.
8. On the Heart Contraction Channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following parameters:
    - Contraction Amplitude, which is the difference between the baseline level of tension in the heart tissue and the tension at the peak of the contraction. To measure this parameter, place one cursor at the beginning of the contraction, and the second cursor on the peak of the contraction. The value for the V2-V1 function on the Heart Contraction Channel is the contraction amplitude. Determine the average amplitude of five consecutive beats.
    - Contraction Period, which is the time between the peaks of two adjacent contractions. To measure this parameter, place one cursor on the peak of a heart contraction, and the other cursor on the peak of an adjacent heart contraction. The value for the T2-T1 function on the Heart Contraction Channel is the contraction period. Determine the average contraction period for five consecutive beats.
9. Record the averaged values in the Journal using the one of the techniques described in Steps 6 or 7, and in Table 1.
10. Scroll to the section of data recorded when cold Ringer's solution was added to the heart. Click AutoScale to maximize the size of the response on the window.
11. Repeat Steps 8, 9 and 10 to measure and record the average contraction amplitude and period of five consecutive beats at the time the cold Ringer's solution was added to the heart and at 30 second intervals for the three minutes after the addition of the cold Ringer's.

12. Repeat Steps 8, 9 and 10 to measure and record the average contraction amplitude and period of five consecutive beats at the end of the recovery period from the effects of cold Ringer's.
13. Determine the heart rate at the times reported in the Journal and on Table 1 by converting the contraction periods to heart rates using the following equation:

$$\text{Heart Rate (BPM)} = \frac{60 \text{ secs/minute}}{(x \text{ seconds/beat})}$$

14. Select Save in the File menu.

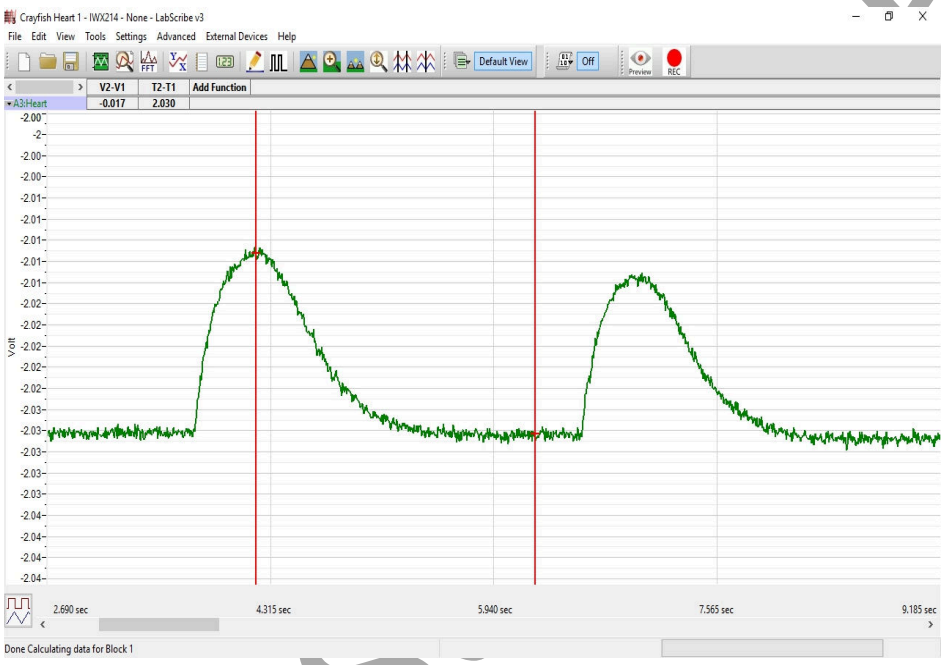

Figure AM-7-L5: Calculating the contraction amplitude using two cursors.

Exercise 3: Drug Effects

1. Scroll to the beginning of the data from Exercise 3 and find the normal heart contractions that occurred before the Serotonin treatment.
2. Use the same techniques used in Exercise 2 to measure the average contraction amplitudes and periods for five consecutive heartbeats during the pre-treatment control, at the end of each treatment (last five beats recorded before changing the concentration or removing the drug), and after recovery. Calculate the heart rate during each period.
3. Record the values for the amplitudes, periods, and heart rates from this exercise in the Journal and on Table 2.
4. Repeat steps 1 and 2 for GABA, recording the values for the amplitudes, periods, and heart rates in the Journal and in Table 3.

Table AM-7-L1: Amplitudes, Periods, and Rate of Heart Contractions at Different Temperatures.

	Contraction		
Treatment	Average Amplitude (V)	Average Period (sec)	Frequency (BPM)
Room Temp Ringer's			
Cold Ringer's			
30 sec later			
60 sec later			
90 sec later			
120 sec later			
150 sec later			
180 sec later			
Recovered from Cold			

Table AM-7-43: Amplitudes, Periods, and Rates of Heart Contraction with Serotonin Treatment.

	Contraction		
Treatment	Average Amplitude (V)	Average Period (sec)	Frequency (BPM)
Pre-Serotonin control			
10^{-6}M Serotonin			
10^{-5}M Serotonin			
10^{-4}M Serotonin			
Recovered			

Table AM-7-L3: Amplitudes, Periods, and Rate of Heart Contraction with GABA Treatment.

	Contraction		
Treatment	Average Amplitude (V)	Average Period (sec)	Frequency (BPM)
Pre-GABA control			
10^{-6}M GABA			
10^{-5}M GABA			
10^{-4}M GABA			
Recovered			

Questions

1. What is the effect of cold Ringer's solution on the rate and the amplitude of the ventricular contraction? What mechanism is responsible for this effect?
2. What effect does Serotonin have on the heart rate and the amplitude of the ventricular contraction? Does the effect vary by dose?
3. If there was a change in the level of the baseline between beats with the introduction of Serotonin, what could cause the change?
4. How does Serotonin produce its effects on the heart rate and the amplitude of the ventricular contraction?
5. What effect does GABA have on the heart rate and the amplitude of the ventricular contraction? Does the effect vary by dose?
6. If there was there a change in the level of the baseline between beats with the introduction of GABA, what could cause the change?
7. How does GABA produce its effects on the heart rate and the amplitude of the ventricular contraction?